Aromatherapy in the Mediterranean Fruit Fly (Diptera: Tephritidae): Sterile Males Exposed to Ginger Root Oil in Prerelease Storage Boxes Display Increased Mating Competitiveness in Field-Cage Trials

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ABSTRACT Previous research showed that exposure to ginger root, *Zingiber officinale* Roscoe, oil increased the mating success of mass-reared, sterile males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). This work, however, involved the exposure of small groups of males ($n = 25$) in small containers (volume 400 ml). Several sterile male release programs use plastic adult rearing containers (so-called PARC boxes; hereafter termed storage boxes; 0.48 by 0.60 by 0.33 m) to hold mature pupae and newly emerged adults before release (≈36,000 flies per box). The objective of the current study was to determine whether the application of ginger root oil to individual storage boxes increases the mating competitiveness of sterile *C. capitata* males. Irradiated pupae were placed in storage boxes 2 d before adult emergence, and in the initial experiment (adult exposure) ginger root oil was applied 5 d later (i.e., 3 d after peak adult emergence) for 24 h at doses of 0.0625, 0.25, 0.5, 1.0, and 2.0 ml. In a second experiment (pupal-adult exposure), ginger root oil was applied to storage boxes immediately after pupal placement and left for 6 d (i.e., 4 d after peak adult emergence) at doses of 0.25 and 1.0 ml. Using field cages, we conducted mating trials in which ginger root oil-exposed (treated) or nonexposed (control) sterile males competed against wild-like males for copulations with wild-like females. After adult exposure, treated males had significantly higher mating success than control males for all doses of ginger root oil, except 2.0 ml. After pupal-adult exposure, treated males had a significantly higher mating success than control males for the 1.0-ml but not the 0.25-ml dose of ginger root oil. The results suggest that ginger root oil can be used in conjunction with prerelease, storage boxes to increase the effectiveness of sterile insect release programs.

KEY WORDS *Ceratitis capitata*, sterile insect technique, ginger root oil, mating competitiveness

THE STERILE INSECT TECHNIQUE (SIT) is an environmentally benign approach for suppressing or eradicating insect pests and is widely used in integrated programs against tephritid fruit flies, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 1995, 2002). The technique involves mass production of males of the target species and release of irradiated (sterilized) males into the environment. Matings between sterile males and wild females yield infertile eggs, which reduces the reproductive potential of the wild population. The success of SIT depends, to a large degree, on the ability of released, sterile males to attract and obtain matings with wild females. This consideration is especially important for species, such as *C. capitata*, in which females display a high degree of mate discrimination, based apparently on male courtship performance (Whittier et al. 1992, 1994).

Unfortunately, the mass-rearing procedures inherent to SIT often lead to a reduction in the mating competitiveness of released *C. capitata* males. Owing to genetic drift and artificial selection in the mass-rearing environment (Leppla and Ozaki 1991), sterile males typically have low mating success relative to wild males (Rossler 1975, Shelly et al. 1994, McInnis et al. 1996, Lance et al. 2000). However, aside from replacing strains frequently, there is currently no effective way to avoid this decrease in mating competitiveness, and for the recently developed genetic sexing strains (e.g., temperature sensitive lethal, *tsl*), such replacement requires considerable time and effort for the development of new and genetically stable translocations (Franz et al. 1996).

Thus, a persistent and important challenge for SIT is the development of simple and inexpensive means to enhance the mating performance of released, sterile *C. capitata* males in the wild. One potentially productive approach involves the prerelease exposure of males to particular attractants. In particular, experiments (Shelly 2001; Shelly and McInnis 2001; McInnis et al. 2002; Shelly et al. 2002, 2003) with both standard (i.e., bisexual) and genetic sexing (i.e., *tsl*) strains have

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demonstrated a strong effect of ginger root, *Zingiber officinale* Roscoe, oil, containing the known male attractant α-copaene; Flath et al. 1994a,b; Nishida et al. 2000) on male mating success. For example, Shelly et al. (2002) examined the effect of ginger root oil on mating competition between sterile males from a *tsl* strain and wild males from Guatemala and Madeira, respectively. A similar result was observed in both cases: the proportion of matings obtained by ginger root oil-exposed (treated) *tsl* males was approximately 2 times greater than that obtained by nonexposed (control) *tsl* males.

Although these studies demonstrated an increase in mating success, exposure to ginger root oil invariably involved small groups of males held in small containers. Specifically, groups of 25 males were held in screen-covered cups (volume 400 ml), and ginger root oil-laden (20 μl) filter paper was placed on the screen. In several SIT programs (most notably, Guatemala and California), sterile pupae and newly emerged adults are held in plastic adult rearing containers (so-called PARC boxes; hereafter referred to as storage boxes; 0.48 by 0.60 by 0.33 m) before release (≈36,000 flies per box). The primary objective of the current study was to determine whether the application of ginger root oil to individual storage boxes increases the mating competitiveness of sterile *C. capitata* males. As described below, two exposure protocols were tested: 1) ginger root oil was applied only after adult emergence in the storage boxes, or 2) ginger root oil was applied when the pupae were introduced into the storage boxes and left until adults were tested. In addition, we assessed whether exposing sterile males to ginger root oil had any effect on their survival in field cages.

Materials and Methods

Flies. Mass-reared males were from a *tsl* strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility (Waimanalo, Oahu, HI). The strain has been mass-reared at the USDA–APHIS facility in El Pino, Guatemala, since 1999, and in 2001 ≈1.25 million eggs from this facility were used to start the colony in Hawaii. Like other *tsl* strains, Vienna-7/Tol-99 possesses a sex-linked mutation, such that treating eggs with high temperature kills all female zygotes, thereby allowing production of males exclusively (Franz et al. 1996). Larvae of the *tsl* mass-reared strain were reared on a standard diet (Tanaka et al. 1969). Males used in the current study were irradiated as pupae 2 d before eclosion in air at 150 Gy of gamma irradiation from a ^{137}Cs source. After irradiation, pupae were placed in six paper bags (100 ml of pupae per bag; 1 ml contains ≈60 pupae), which, in turn, were placed inside individual storage boxes. Most adult emergence occurred 2 d after pupal placement, and emerging *tsl* males were fed a sugar-agar gel placed on a screened opening on top of the box. Most of the tests described herein were conducted in mid-2002; consequently, the *tsl* strain had been mass-reared for ≈45 generations (13 generations per year for 3.5 yr) before our study.

Because wild flies were not available in sufficiently large numbers, we used "wild-like" flies for most of the mating trials. These flies derived from a laboratory colony started with 200–300 adults reared from Jerusalem cherry, *Solanum capsicum* L., collected in Hawaii Volcanoes National Park. Adults were held in screen cages and provided with a sugar-protein (yeast hydrolysate) mixture (3:1 by weight), water, and an oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval diet in plastic containers over vermiculite for pupation. Adults used in the mating trials were separated by sex within 24 h of eclosion, well before reaching sexual maturity at 5–7 d of age (T.E.S., unpublished data) and kept in screen-covered buckets (5-liter volume; 100–125 flies per bucket) with ample food (sugar-protein mixture) and water. When used in the current study, the wild-like flies were seven to 11 generations removed from the wild.

To confirm the results obtained using wild-like flies, selected experiments were repeated using wild flies reared from coffee, *Coffea arabica* L., collected on Kauai. Fruits were transported to the laboratory and placed over vermiculite, and larval development proceeded in situ. Pupae were sifted from the vermiculite 7–10 d later, and adults were separated by sex within 48 h of eclosion (wild flies attain sexual maturity at 7–10 d of age; T.E.S. and D.O.M, unpublished data). Wild flies were maintained using the protocol described above for wild-like flies. Adults of all strains used in the study were maintained at 20–24°C and 65–85% RH and received both natural and artificial light with a photoperiod of 12:12 (L:D) h.

Exposure to Ginger Root Oil. Ginger root oil, which was obtained from Citrus and Allied Essences (Lake Success, NY), contains α-copaene (a hydrocarbon sesquiterpene) in low concentration (0.4%, T. W. Phillips, personal communication) with the positive enantiomer predominating (81%; Takeoka et al. 1990). Ginger root oil contains additional sesquiterpenes, but their effect on *C. capitata* either independently or in combination with α-copaene remain largely unknown (but see Flath et al. 1994a,b).

We exposed *tsl* males to ginger root oil in two different ways. For "adult" exposure, ginger root oil was applied to the storage box for a 24-h period starting 3 d after the day of peak adult emergence. For "pupal-adult" exposure, ginger root oil was applied to the storage box immediately after placement of the pupae in the box and left there for 6 d. Thus, in both cases, exposure was terminated when *tsl* males were 4 d old. Note that "pupal" exposure was not investigated, because 1) earlier results (Shelly 2001) showed that exposure of pupae only (and not the subsequently emerged adults) to ginger root oil did not influence male mating competitiveness; and 2) operationally, removal of the oil before "harvesting" the flies for release would create extra work and therefore would probably not be implemented in SIT programs. Five doses of ginger root oil (0.0625, 0.25, 0.5, 1.0, and 2.0

ml) were tested for adult exposure, and two doses (0.25 and 1.0 ml) were tested for the pupal-adult exposure. Using a pipette, we applied ginger root oil to 10-cm squares of blotter paper for doses >0.25 ml and to 5-cm squares of blotter paper for doses ≤0.25 ml. The oil-laden paper was placed on the screened opening on the top of the storage box (not touching the food gel), because previous work (Shelly 2001) showed that exposure to the oil's aroma alone (i.e., without direct contact with the oil) conferred a mating advantage (in fact, when given access to ginger root oil, males do not feed on it but instead become quiescent). An empty storage box was then placed on the treated box to mimic SIT programs, where storage boxes are stacked to save space. For each storage box set up with ginger root oil, we also set up a storage box that received the same quantity of pupae but no ginger root oil, thus yielding nonexposed (control) *tsl* males. In all instances, boxes receiving the ginger root oil treatment were kept in a separate room from those not receiving the treatment to prevent inadvertent exposure of control *tsl* males.

Immediately after exposure, we removed one paper bag (and the males resting on them) from the storage box, quickly transferred them to screen cages (30-cm cubes), and gently shook them to disperse the males. In the majority of mating trials, *tsl* males were used the following day (i.e., when 5 d old). For a given trial, we marked either the wild-like or the *tsl* males, alternating the marked group between successive trials. Males were marked 1 d before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of handling. After marking, males were held in plastic buckets with ample food (sugar-agar gel for *tsl* males and the sugar-protein mixture for wild-like males). Control *tsl* males were removed from the storage box on the same day as the associated treated males and handled in the same manner.

Mating Trials. Mating trials were conducted at the Agricultural Experiment Station of the University of Hawaii, Waimanalo, between January and June 2002. Groups of 100 wild-like males, 100 wild-like females, and either 100 treated *tsl* males or 100 control *tsl* males were released between 0800 and 0830 hours in field cages (height, 2.5 m; diameter, 3.0 m) that contained two artificial trees (each 2 m in height with ≈450 leaves resembling those of *Ficus benjamina* L.). Artificial trees were used, because the available host trees (e.g., common guava, *Psidium guajava* L., and orange, *Citrus sinensis* L) contain chemicals (α-copaene among them) in their bark and leaves that affect male sexual behavior (Shelly and Villalobos 2004; T.E.S., unpublished data). Artificial trees, however, provided a chemically neutral substrate on which the flies displayed the entire complement of natural activities. The cages were monitored for 3 h, mating pairs were collected in vials, and the males identified. On most test days, we ran two or four trials (one-half with control *tsl* males and one-half with treated *tsl* males for a given experiment), and only trials in which 20% of the females mated were included in the analysis. Over the study period, air temperature ranged between 25 and 30°C during the trials. When tested, wild-like males and females were 7–11 d old and 8–13 d old, respectively.

Secondary Mating Experiments. As noted below, exposure to ginger root oil did, in most instances, increase the mating success of *tsl* males. To more rigorously identify and assess this enhancement, we conducted three additional experiments following the same basic procedures described above. First (experiment A), we performed mating trials by using wild flies instead of wild-like flies. In these trials, treated *tsl* males were exposed to a single dose of ginger root oil (1 ml) for the adult and pupal-adult exposure regimes, respectively. Also, owing to the low availability of wild flies, these trials were run using 75 wild flies of each sex and 75 *tsl* males. When tested, wild males and females were 9–15 d old and 10–16 d old, respectively. Second (experiment B), after removal from the storage box, *tsl* males (both control and treated) were held 3 d before use in mating trials (with wild-like flies) to determine whether exposure to ginger root oil conferred a mating advantage for intervals >1 d. These trials were performed using a single dose (1 ml) of ginger root oil for the adult exposure regime only. Third (experiment C), we investigated the effects of cooling on the mating success of treated *tsl* males. In Mediterranean fruit fly control programs, *tsl* males are cooled for transfer and storage in release aircraft and may remain cooled for several hours before release. Two storage boxes were set up and treated with 1 ml of ginger root oil for adult exposure. Immediately after exposure, one box was placed in a walk-in refrigerator (4°C) for 3 h, whereas the other remained at room temperature. Males were then marked as described above and tested the next day (using wild-like flies).

Effect of Ginger Root Oil on Survival. The effect of ginger root oil on male survival was tested by releasing control and treated *tsl* males in field tents containing rooted guava trees, *Psidium guajava* L., and scoring the number of survivors after a 2-d interval. Treated *tsl* males were exposed to a single dose (1 ml) of ginger root oil for adult exposure and pupal-adult exposure, respectively, after the above-mentioned procedures. Groups of 100 control and 100 treated *tsl* males were placed into field cages when 5 d old. As with the mating trials, we marked only males from one group (i.e., control or treated) and alternated the identity of the marked group between successive trials. Marking procedures followed those described above. After 2 d, we searched the tents intensively and collected males by gently coaxing them into vials; searching typically lasted 45–60 min per tent. Tests were run using two field tents; the enclosed trees bore no fruit or flowers during the tests, and no food or water was added to the tents.

Statistical Analyses. Pairwise comparisons were made using the *t*-test, and multiple comparisons were made using analysis of variance (ANOVA), followed by the Tukey test where significant variation was detected. Proportions were arcsine transformed for anal-

Table 1. Number of matings obtained by treated (exposed to ginger root oil) and control (nonexposed) *tsl* males in competition with wild-like males for wild-like females, for the adult exposure regime, at different doses of ginger root oil

Dose (ml)	Competing males	Matings	*t*
0.0625	Treated *tsl*	18.4 (2.1)	
	Wild-like	24.2 (2.4)	1.1^{NS}
	Control *tsl*	10.1 (1.2)	
	Wild-like	29.4 (1.7)	6.1***
0.25	Treated *tsl*	16.8 (1.7)	
	Wild-like	15.7 (1.8)	0.4^{NS}
	Control *tsl*	8.2 (0.9)	
	Wild-like	22.1 (1.6)	6.8***
0.5	Treated *tsl*	32.2 (2.1)	
	Wild-like	24.8 (2.4)	2.3*
	Control *tsl*	14.9 (1.8)	
	Wild-like	42.8 (2.2)	9.7***
1.0	Treated *tsl*	28.1 (2.5)	
	Wild-like	25.5 (3.0)	0.7^{NS}
	Control *tsl*	11.6 (1.9)	
	Wild-like	37.6 (2.1)	9.1***
2.0	Treated *tsl*	18.9 (3.3)	
	Wild-like	33.9 (3.3)	3.2**
	Control *tsl*	11.6 (1.7)	
	Wild-like	34.6 (3.1)	6.5***

One hundred females and 100 males of each type were used per replicate. Values represent the mean number of matings per replicate; standard error is given in parentheses. Where the *t*-test revealed a significance difference in matings between *tsl* and wild-like males, the male type with the larger mean is in bold type. For a given dose, 14 replicates were conducted for trials involving treated and control *tsl* males, respectively (df = 26 in all *t*-tests).

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

ysis. Data met the assumptions of normality and homoscedasticity for all tests. All analyses were performed using SigmaStat Statistical Software (version 2.0).

Results

Mating Competitiveness: Adult Exposure. For adult exposure, exposure to ginger root oil had a major effect on the mating success of *tsl* males relative to wild-like males (Table 1). For all doses of ginger root oil <2.0 ml, treated *tsl* males achieved equivalent or (for one dose, 0.5 ml) even greater numbers of matings than the wild-like males. In contrast, control males obtained significantly fewer matings, on average, than wild-like males in all cases. Interestingly, the maximum dose tested (2.0 ml) had a negative effect on the mating success of treated *tsl* males, and, after exposure to this dose, treated *tsl* males actually accounted for significantly fewer matings than wild-like males.

The difference in mating competitiveness between treated and control *tsl* males is particularly evident when their relative mating success (percentage of total matings) is compared over different doses of ginger root oil (Fig. 1). For doses <2.0 ml, the relative mating success of treated *tsl* males was relatively constant over the different doses, with mean values ranging from 47 to 56%. Among the four doses <2.0 ml, we found that dosage had no significant effect on relative mating success of treated *tsl* males ($F_{3, 52} = 1.9$; one-way ANOVA; $P > 0.05$). For doses <2.0 ml, the associated mating trials conducted using control *tsl* males revealed that these males also accounted for a fairly constant proportion of the total matings, but mean values were much lower and ranged from 23 to 27%. On average, there was no significant variation in the relative mating success of control *tsl* males tested in association with different doses of ginger root oil ($F_{4, 65} = 1.0$; one-way ANOVA; $P > 0.05$). As these data suggest, for doses of ginger root oil <2 ml, a two-way ANOVA revealed that male type (control or treated) accounted for a significant amount of the observed variation in relative mating success ($F_{1, 104} = 166.1$; $P < 0.001$), but dosage did not ($F_{3, 104} = 2.0$; $P > 0.05$). The male type × dosage interaction was not significant ($F_{3, 104} = 1.3$; $P > 0.05$).

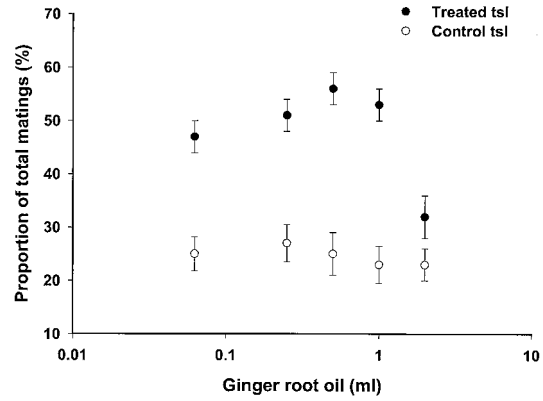

Fig. 1. Relative mating success of treated (exposed to ginger root oil) and control (nonexposed) *tsl* males over different doses of ginger root oil for adult exposure. Mean values are plotted (±1 SE); $n = 14$ replicates in all cases. The ordinate represents the proportion of matings obtained by treated or control *tsl* males in competition against wild-like males for copulations with wild-like females. Note that values (doses) on the abscissa are plotted on a $\log_{10}$ scale.

In contrast to the smaller doses, application of 2.0 ml of ginger root oil had little effect on the relative mating success of *tsl* males (Fig. 1). The mean proportion of matings obtained by treated males was 32% compared with 23% for the associated control *tsl* males ($t = 1.3$, df = 26, $P > 0.05$). When the 2.0-ml dose was included in a one-way ANOVA, significant variation in relative mating success of treated *tsl* males was detected among the different doses ($F_{4, 65} = 7.5$; $P < 0.001$), with the values for all lower doses differing significantly from the 2.0-ml dose ($P < 0.05$ for all comparisons; Tukey test) but not from one another ($P > 0.05$ for all comparisons; Tukey test). Because of this result, a two-way ANOVA by using data from all doses of ginger root oil had a significant male type × dosage interaction ($F_{4, 130} = 5.2$; $P < 0.001$).

Mating Competitiveness: Pupal-Adult Exposure. For pupal-adult exposure, the 0.25-ml dose of ginger root oil had no effect on the mating success of treated *tsl* males relative to wild-like males (Table 2). Wild-like males obtained significantly more matings, on average, than treated or control *tsl* males, and the

Table 2. Number of matings obtained by treated (exposed to ginger root oil) and control (nonexposed) *tsl* males in competition with wild-like males for wild-like females, for the pupal-adult exposure regime, at two doses of ginger root oil

Dose (ml)	Competing males	Matings	*t*
0.25	Treated *tsl*	13.8 (1.7)	
	Wild-like	22.9 (1.6)	3.5**
	Control *tsl*	12.5 (1.0)	
	Wild-like	25.2 (1.6)	5.0***
1.0	Treated *tsl*	22.5 (2.0)	
	Wild-like	24.9 (2.4)	0.7^{NS}
	Control *tsl*	15.6 (2.6)	
	Wild-like	33.6 (2.1)	5.7***

One hundred females and 100 males of each type were used per replicate. Values represent the mean number of matings per replicate; standard error is given in parentheses. Where the *t*-test revealed a significance difference in matings between *tsl* and wild-like males, the male type with the larger mean is in bold type. For a given dose, 14 replicates were conducted for trials involving treated and control *tsl* males, respectively (df = 26 in all *t*-tests).

Significance levels: ** $P < 0.01$, *** $P < 0.001$.

Table 3. Results of secondary mating experiments measuring the competitiveness of treated (exposed to ginger root oil) and control (nonexposed) *tsl* males (A) against wild males for wild females and (B) 3 d after exposure

Experiment	Exposure	Competing males	Matings	*t*
A	Adult	Treated *tsl*	17.8 (1.1)	
Wild flies		Wild	7.5 (1.7)	5.1***
		Control *tsl*	7.0 (1.9)	
		Wild	18.1 (2.4)	3.7**
	Pupal-Adult	Treated *tsl*	11.3 (1.1)	
		Wild	11.7 (1.6)	0.2^{NS}
		Control *tsl*	6.7 (1.2)	
		Wild	17.1 (2.2)	5.5***
B	Adult	Treated *tsl*	23.5 (2.1)	
3-d postexposure		Wild-like	24.6 (2.2)	0.3^{NS}
		Control *tsl*	13.6 (1.4)	
		Wild-like	36.5 (1.8)	8.4***
C	Adult	Cooled *tsl*	25.5 (4.6)	
Cooling		Wild-like	22.2 (3.3)	0.6^{NS}
		Non-cooled *tsl*	26.2 (4.2)	
		Wild-like	22.3 (2.2)	1.4^{NS}

In assessing C, the effects of cooling, all *tsl* males were exposed to ginger root oil, and relative mating success was measured for cooled versus noncooled males. Wild-like males and females were used in the two latter experiments; a dose of 1.0 ml of ginger root oil was used in all cases. Seventy-five females and 75 males of each type were used per replicate in experiment A, and 100 females and 100 males of each type were used per replicate in experiments B and C. Values represent the mean number of matings per replicate; standard error is given in parentheses. Where the *t*-test revealed a significance difference in matings between *tsl* and wild-like males, the male type with the larger mean is in bold type. For a given experiment and exposure regime, six replicates were conducted for trials involving treated and control *tsl* males, respectively (df = 10 in all *t*-tests).

Significance levels: ** $P < 0.01$, *** $P < 0.001$.

mean number of matings obtained by treated and control *tsl* males was not significantly different ($t = 0.7$, df = 26, $P > 0.05$). Likewise, for the 0.25-ml dose, there was no significant difference between the proportion of total matings achieved by treated (mean = 35; SE = 4.4; $n = 14$) and control (mean = 33, SE = 2.8, $n = 14$) *tsl* males ($t = 0.3$, df = 26, $P < 0.05$). However, at the 1.0-ml dose, treated *tsl* males achieved approximately the same number of matings as wild-like males, whereas the associated control *tsl* males were outcompeted by wild-like males. At this higher dose, treated *tsl* males (mean = 46%, SE = 4.4, $n = 14$) accounted for a significantly greater proportion of total matings than did control *tsl* (mean = 31%, SE = 2.7, $n = 14$) males ($t = 2.9$, df = 26, $P < 0.01$). For the 1.0-ml dose, there was no significant difference in the relative mating success of treated *tsl* males from the adult and pupal-adult exposure regimes ($t = 1.2$, df = 26, $P > 0.05$).

Additional Mating Experiments. An increase in the mating competitiveness of treated *tsl* males was also detected when wild flies were used instead of wild-like flies (Table 3A). In the trials involving adult exposure, the treated *tsl* males obtained significantly more matings per replicate than wild males. At this dose (1.0-ml dose), treated *tsl* males actually accounted for a significantly greater proportion of the total matings when competing with wild males than with wild-like males ($t = 3.4$, df = 18, $P < 0.01$). In contrast, control *tsl* males obtained significantly fewer matings per replicate than wild males. Control males obtained about the same proportion of the total matings when competing with wild males as with wild-like males (using data from control males associated with 1.0 ml dose; $t = 0.5$, df = 18, $P > 0.05$).

In the trials involving pupal-adult exposure, treated *tsl* and wild males had equivalent mating success. At the 1.0-ml dose, treated *tsl* males accounted for similar proportions of the total matings when competing with wild males than with wild-like males ($t = 0.5$, df = 18, $P > 0.05$). In contrast, control *tsl* males obtained significantly fewer matings than wild males. Control males obtained about the same proportion of the total matings when competing with wild males as with wild-like males (using data from control males associated with 1.0-ml dose; $t = 1.6$, df = 18, $P > 0.05$).

When tested 3 d after adult exposure, treated *tsl* males obtained similar a number of matings per replicate as wild-like males, and control *tsl* males had a significantly lower mating frequency than wild-like males (Table 3B). At the 1.0-ml dose, the proportion of matings obtained by treated *tsl* males 3 d after exposure was not significantly different from that obtained by treated *tsl* males 1 d after adult exposure ($t = 0.3$, df = 18, $P > 0.05$).

Cooling *tsl* males after exposure seemed to have no effect on their mating success (Table 3C). On average, treated (cooled) and control (noncooled) *tsl* males accounted for 52 and 54% of the total matings, respectively ($t = 0.2$, df = 10, $P > 0.05$).

Survivorship. The mean number of survivors did not differ significantly between the two trees for the treated or control males for the adult or pupal-adult exposure regimes, and consequently data were pooled across the trees (*t*-test; $P > 0.05$ in all tests). Exposure to ginger root oil had no apparent effect on male survival after adult or pupal-adult exposure. For adult exposure, 46.5 ± 2.9 treated *tsl* males survived, on average, compared with 48.2 ± 3.8 for control *tsl* males ($t = 0.3$, df = 22, $P > 0.05$). For pupal-adult exposure,

49.3 ± 4.8 treated *tsl* males survived, on average, compared with 49.7 ± 3.4 for control *tsl* males ($t = 0.1$, df = 22, $P > 0.05$).

Discussion

The current study shows that, at certain doses, the addition of ginger root oil to prerelease storage boxes increases the mating success of mass-reared, sterile *tsl* males of *C. capitata* in field-cage tests. The oil was effective over a wide range of doses (0.0625–1.0 ml) when applied after adult emergence. Over this range, the relative mating success of treated (exposed) *tsl* males was ~2 times that of control (nonexposed) *tsl* males. However, no increase in mating competitiveness was detected at the highest dose (2 ml) of the adult exposure. When applied at the time of pupal placement (and left in place throughout adult emergence), ginger root oil enhanced mating frequency at the 1.0-ml dose but not the 0.25-ml dose. It thus seems that, owing to volatilization before adult emergence, pupal-adult exposure requires relatively higher doses of ginger root oil than adult exposure to effect an increase in mating frequency. At a dose of 1.0 ml, the relative mating success of treated *tsl* males from the pupal-adult exposure was similar to that observed for treated *tsl* males from the adult exposure. Although only a single dose was tested (1.0 ml), trials using wild flies generated the same findings as trials using wild-like flies for both adult and pupal-adult exposure, signaling robustness in the data.

Evidence for enhanced mating success, of course, does not constitute definitive proof that exposure to ginger root oil will increase the effectiveness of SIT in field conditions. For example, even though treated *tsl* males have heightened competitive ability, they may not fully inhibit female remating (presumably owing to inadequate transfer of sperm or accessory gland products; Vera et al. 2003; but see McInnis et al. 2002, Shelly and Kennelly 2002, and Shelly et al. 2002 for contradictory results), and wild females may eventually mate with wild males and produce fertile eggs. The strongest support would likely derive perhaps from repeated field releases of control or treated *tsl* males in designated areas with long-term, periodic monitoring of egg hatch (induced sterility), fruit infestation, and wild fly abundance in areas receiving a given type of male. Such studies are currently planned for Guatemala and Hawaii. Nonetheless, obtaining matings with wild females represents perhaps the greatest constraint on the effectiveness of sterile males, and the enhanced mating success described here fuels strong optimism that prerelease exposure to ginger root oil will, in fact, lead to heightened suppression of wild flies.

As noted previously, Shelly and Villalobos (2004) found that males exposed to natural sources of α-copaene (specific sites on branches of guava trees as well as guava and orange fruits) gained a mating advantage over nonexposed males. These tests were conducted with wild flies exclusively, and it is not known whether exposure to such sites would likewise confer a mating advantage to mass-reared *C. capitata* males. If so, the performance of *tsl* males may actually be enhanced through natural exposure to particular plant products. Even so, prerelease exposure to ginger root oil eliminates the "need" for sterile males to locate chemical sources in the environment (thereby eliminating time and energy costs associated with searching) and guarantees that sterile males benefit fully from exposure to a performance-enhancing essential oil.

Relative to other costs incurred in SIT programs, the use of ginger root oil on individual storage boxes would represent only a minor expense. For example, the California Department of Food and Agriculture in conjunction with USDA–APHIS obtains sterile, *tsl* pupae from a facility in Guatemala, maintains males for several days posteclosion and then aerially releases ≈221 million males (or ≈7,300 storage boxes) per week over southern California (E. Y. Smith, personal communication). If 0.50 ml of ginger root oil is applied to each box, then the cost of ginger root exposure (using 3.65 liters of the oil) would be about $264 per week (1 liter = $72.50; Citrus Allied and Essences) or, in terms of male numbers, ~$1.19 per million males released. This expense is minor compared with the purchase and delivery of the pupae, which collectively cost approximately $179 per million males (E. Y. Smith, personal communication). Prerelease exposure to ginger root oil would involve additional tasks (e.g., preparing dispensers, applying the oil), but these are simple, quickly completed tasks that would not require much additional labor. It is also possible that ginger root oil could be included in the agar food blocks placed on the storage boxes. In addition, financial and labor costs associated with preexposure to ginger root oil could be greatly reduced if entire rooms, containing dozens of storage boxes, could be aromatized. Tests examining the effectiveness of a "whole room" exposure regime are planned for Hawaii.

In addition to its comparatively low cost, the increase in the mating competitiveness of released males would possibly permit reduction in the quantity of flies released without any decrease in the program's effectiveness. For example, using field cages, Barry et al. (2003) found that *tsl* males exposed to ginger root oil and released at a 1:1 ratio with wild males obtained the same number of matings with wild females as nonexposed *tsl* males released at a 10:1 ratio with wild males. Reducing the number of flies released could result in substantial cost savings for SIT programs. For example, a 50% reduction in release volume would cut ~$1 million from the annual budget of the California program.

In conclusion, although a positive effect of ginger root oil on the mating success of male Mediterranean fruit flies has been demonstrated in several studies, the behavioral or physiological mechanisms responsible for this phenomenon remain unknown. Exposure to ginger root oil does seem to increase pheromone-calling among males (Shelly 2001). However, this increase seems too small to account for the large differences observed in the relative mating success of

treated and control males. In addition, experiments conducted both in the field (Shelly 2001) and in a wind tunnel (N. Papadopoulos and T.E.S., unpublished data) fail to reveal any differences in female attraction to the sex pheromone of treated versus control males. Analysis of courtship behavior using videotape (D. Briceno and T.E.S., unpublished data) suggests that wild females more readily "accept" or "cooperate" with males exposed to ginger root oil. The durations of premounting activities, such as wing vibration and buzzing and head rocking, were lower for ginger root oil-exposed males than control males. Whether this finding reflects differences between treated and control males in the rate and/or form of certain courtship displays or in close-range olfactory cues (e.g., "perfumed" versus "normal" male exoskeleton) remains unknown.

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